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UNITED STATES
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DEPARTMENT CIRCULAR 299

Washington. D. C. January, 1923

QUARANTINE PROCEDURE TO SAFEGUARD THE INTRODUCTION OF CITRUS PLANTS:

A SYSTEM OF ASEPTIC PLANT PROPAGATION.

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INTRODUCTION.

PLANT BREEDING, to be of maximum efficiency, must deal with all existing wild relatives of the plant being bred. Only in this way may investigators avail themselves of disease resistance or other special advantages acquired through ages of evolutionary progress.

The dangers attending the introduction of foreign plants have become so patent in recent years that it may well cause us to pause to count the cost and consider whether the benefits sought are commensurate with the risk involved. Numerous as are the pests already lodged with us as a result of miscellaneous unsupervised plant introductions, others even more threatening are known to exist in foreign countries. If we are to continue the introduction of foreign plants so necessary for study, testing, and breeding purposes, it is apparent that improved technique must be employed in the handling of such introductions.

At the Toronto meeting of the American Society of Agronomy, held December 29, 1921, Dr. Karl F. Kellerman presented a paper[1] detailing some of the precautions considered important in handling imported citrus plants to guard against introducing dangerous new pests and diseases. Special equipment for greenhouses intended for growing citrus plants in quarantine and special procedure in handling such plants were described and emphasized as requisite precautionary measures. The equipment and procedure for handling citrus plants have been further developed and perfected since that date, so that it seems desirable to record and describe the methods at present followed in the citrus quarantine house of the United States Department of Agriculture.

While the procedure for handling citrus plants is perhaps more rigorous than would commonly be thought necessary, in essential outlines it is easily adaptable; and the equipment, especially as it guards against insect or fungous infestation, could readily be applied to any well-built greenhouse. The illustrations accompanying this circular will assist in making this plain.

PROTECTION BY FUMIGATION AND SPRAYING.

About 1911, following a program drawn up by the senior writer, a concerted effort was made to grow citrus plants in the Washington greenhouses free from the attacks of insects, such as ants, red spiders, scale, mealybug, and mites. The program included fumigation twice each month and careful inspection of plants, with occasional spray applications.

In practice, this system gave a considerable measure of relief from damage, but eradication was not complete, and the treatments were sometimes necessarily so drastic as to injure the plants. Ants in particular were able to escape the effect of fumigation by nesting in the soil. With them the eggs and larvæ of other pests were doubtless preserved.

In addition to the actual damage caused by insects, it was apparent that insects, particularly ants, are the active spreaders of fungous and bacterial diseases. Therefore it was finally realized that the greenhouse must be so constructed that it would be insect proof.

PROTECTION THROUGH SPECIAL DEVICES FOR THE EXCLUSION OF INSECTS.

The first step in providing equipment for protecting the citrus plants grown in the greenhouse from insect attacks was the construction of a propagating bed for growing citrus seedlings. This bed was of sheet-metal construction and was surrounded by a water-filled trough or moat, primarily intended to exclude ants. The success of this device, which was first used in experimental greenhouse work by Dr. K. F. Kellerman in 1906, encouraged more complete efforts at protecting the greenhouse benches.

A further advance in the campaign was made in 1915 when a screened cage was built in the end of one of the greenhouses, the bottom and sides being made entirely of metal, like a huge pan, and a moat for holding water or oil provided entirely around the cage. This was designed as a detention cage to separate any suspicious plants from the rest of the greenhouse. Figure 1 shows how this screened cage looks in actual use.

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[1] Kellerman, Karl F., and Swingle, Walter T. (Joint paper.) Eradication of citrus canker and safeguarding the citrus industry against recurring epidemics.

Later, this cage was found of service as a temporary detention compartment for plants which had passed through the regular quarantine procedure put into practice as a result of the menace of citrus canker.

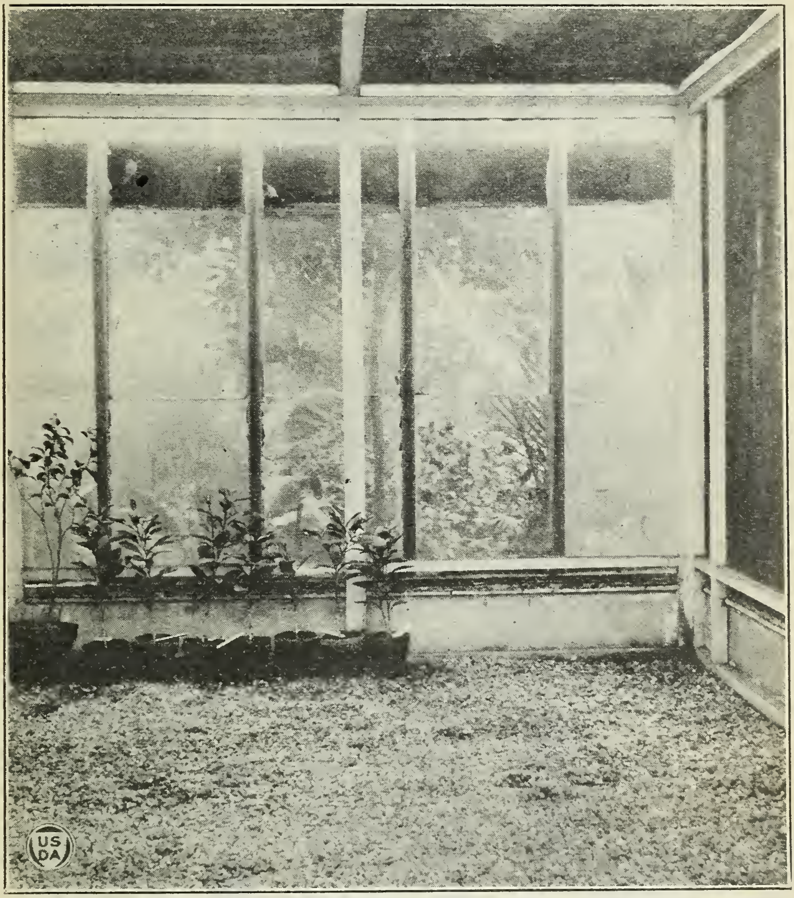

Fig. 1.—Screened detention cage surrounded by an oil moat.

In 1916 the interception of some imported citrus budwood of a suspicious character but of a promising new variety made it necessary to provide a propagating house entirely removed from all other citrus plants. Accommodations were secured through cooperative arrangement with the Bureau of Animal Industry on the grounds of its station at Bethesda, Md., near Washington, D. C., and a small temporary glass-roofed shed was erected for use in propagating imported citrus plants. This first house was only 12 feet long by 6 feet wide and 4½ feet high. It was heated by gas, thermostatically controlled, using a hot-water coil. As the first plants grown here were from citrus-canker territory, great precautions were observed in the sterilization of clothing and tools, and a system of aseptic propagation was gradually evolved.

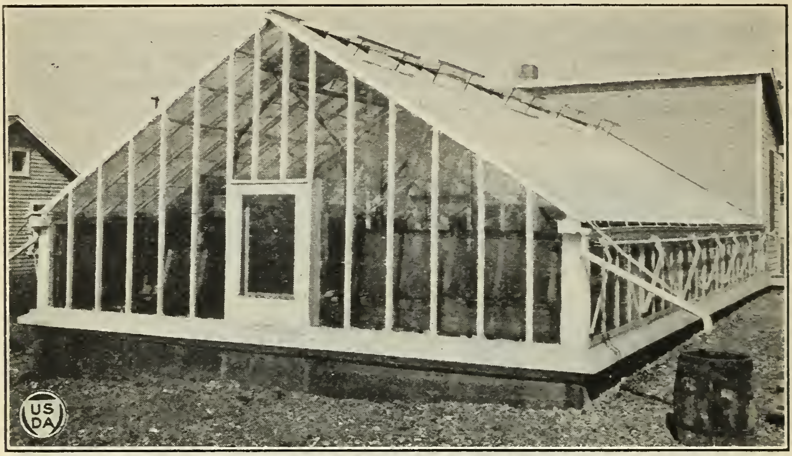

FIG. 2.—End view of the citrus quarantine greenhouse at Bethesda, Md. This is the receiving end, or isolation ward. The exterior oil moat is attached to the foundation wall just below the sill.

In 1918 a small but well-equipped greenhouse was built at Bethesda, taking the place of the temporary propagating shed just described and providing more adequate space for both plants and workers. Views of this house are shown in Figures 2 and 3. The original propagation shed is shown in Figure 4. The greenhouse is approximately 35 feet long by 20 feet wide, with a service room 10 by 20

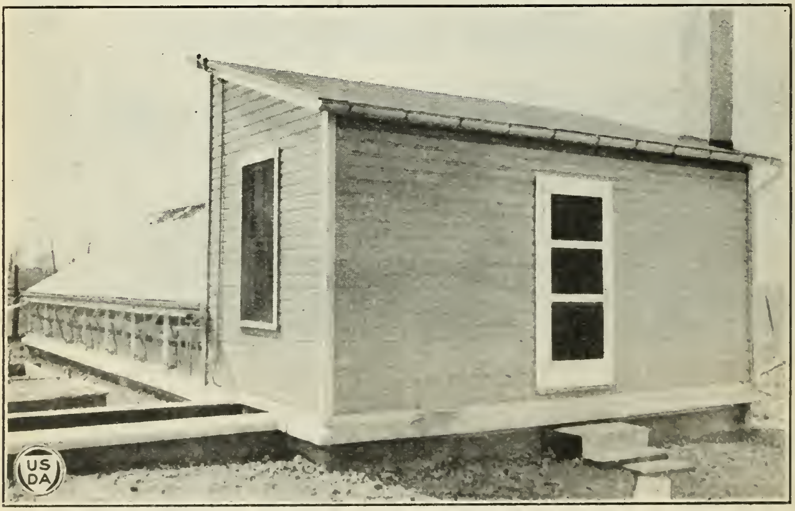

FIG. 3.—Citrus quarantine greenhouse, showing all openings screened and entrances protected by a circumferential oil moat.

feet. It is divided into two sections, the end section serving as a receiving room, or isolation ward, for newly imported plants (fig. 2). In this greenhouse were incorporated all the insect-proof devices that had previously been tested and found satisfactory and a number of new ones.

OIL CUPS.

At this time Dr. B. T. Galloway and Edward Goucher, who had been working along similar lines for the control of insects in the Federal Horticultural Board greenhouse, perfected an oil cup designed to be attached to the supporting legs of the greenhouse benches, which was effective in keeping crawling insects from gaining access to the bench proper.

This device, saucer shaped, was cast in molds, with an opening in the center large enough to fit tightly over the pipe serving as a bench leg to which it was to be attached. As castings could not always be obtained promptly, a similar cap was designed by Eugene May, jr., using heavy galvanized sheet metal and soldering this to a metal sleeve to slip snugly over the iron pipe used as a support for the bench. Any packing necessary was saturated with oil to repel insects that might try to elude the oil cup itself. These sheet-metal cups were used on the bench supports of the new quarantine greenhouse at Bethesda.

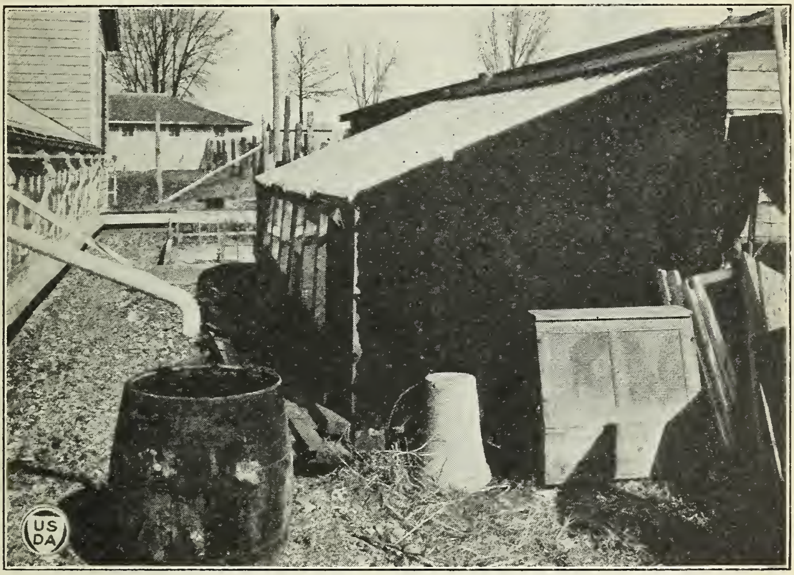

FIG. 4.—Original propagating shed temporarily used as a quarantine greenhouse for plants originating in the citrus-canker territory of the Orient.

This form of oil cup can be made by any sheet-metal worker at small expense and can be attached to benches already in service without necessitating any changes in construction. The cup is shown in Figure 5.

A heavy oil, such as cup grease, can be used in such cups to advantage, as the evaporation is negligible and there is less likelihood of its being washed out in watering plants than when using a lighter oil. An improved form of the cup with a projecting cover obviates all danger of the oil being washed out, but this adds considerably to the cost.

For such devices to be effective, the plant bench must be entirely separated from the side walls of the greenhouse. In addition, at the Bethesda greenhouse, screens have been put up along the backs of the benches (fig. 6) to prevent the branches of growing plants from touching the side wall of the house. Ants will quickly take advantage of any such bridge and find their way to the bench.

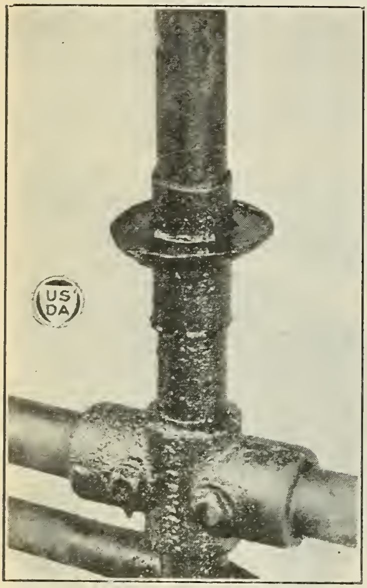

Fig. 5.—Metal oil cup attached to the leg of a greenhouse bench to prevent the access of crawling insects.

CONCRETE FLOOR.

Floors were constructed entirely of concrete. A concrete floor that may be easily cleaned with a hose and drained through an insect-proof trap is a distinct advantage in keeping the greenhouse free from crawling insects.

EXTERIOR OIL MOAT.

In quarantine work it is not only essential to keep insects off the benches, but the escape of any that may gain entrance to the greenhouse with imported plants must be prevented. To make doubly sure of safety on this score, the citrus quarantine house, at the suggestion of Doctor Kellerman, was provided with an oil moat or trough that extends entirely around it, about a foot above the ground (figs. 2 and 3). The U-shaped trough was fastened to the cement foundation wall by means of expansion bolts, a piece of oil-soaked felt packing between the wall and the trough effectively preventing any insects from eluding the oil barrier. A removable cover (fig. 7) permits inspection and cleaning and excludes rainwater, while it prevents sticks or other trash from accumulating and bridging the barrier. The oil moat shown in the illustration holds nearly a barrel of crude oil and needs only occasional inspection to be fully effective.

SCREENED VENTILATORS.

The features already described eliminated danger from crawling insects, but the flying ones remained to be considered. Doors were, of course, easily screened, but the ventilators offered a difficult problem, owing to the arms by which they are operated. An ingenious device of simple construction was finally invented[2] consisting of a sliding gasket fastened to the screen wire, which permits the operating arm to pass through the screen with a free movement and yet leaves no opening for the entrance of insects (fig. 8).

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[2] Patent No. 1,402,241, issued Jan. 3, 1922, to Eugene May, jr., Robert J. Bullen, and August Voight, assistants in the plant-propagation work of the Office of Crop Physiology and Breeding Investigations.

A Government patent covering this invention has been taken out, which renders it available without the payment of royalties to those who may wish to use it in making greenhouses insect proof. The detail drawings (fig. 9) and descriptive text will explain haw this device is constructed. In the first models the gasket was held in place by metal straps which were soldered to the sliding member, but this attachment is now made secure by the use of small screw bolts, which facilitate the replacing of the gaskets when necessary.

The device consists of a ventilator arm (*A*) of ordinary type and a trap, as shown in Figure 9. The trap consists of a body of sheet metal the ends of which are folded over (*D*) about 1 inch, forming an envelope. Through the body a rectangular opening, about 3 by 2 inches, is provided. Over this opening is placed a felt gasket with a slot in the center, the gasket being fastened by straps held in place by screw bolts or soldered to a flat piece of metal also provided with a slot in the center suitable in size and shape to accommodate the ventilator arm. This piece of metal is free to slide in the outer envelope body, allowing free movement of the ventilator arm. One of the ends of the body is bent down at an angle and holes are provided for fastening it to the greenhouse header or roof. At the upper end the body is soldered to the wire screen through which a slit is provided to permit the passage of the ventilator arm. Thus the ventilator opening is completely screened, preventing the access of insects when the ventilators are raised.

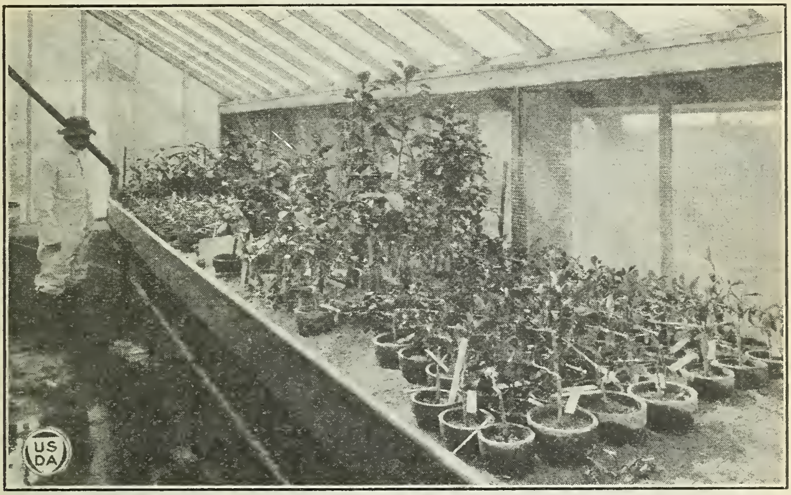

Fig. 6.—Interior of citrus quarantine greenhouse, showing metal construction and screen at the back of the bench to prevent the growing plants from touching the side wall.

Another unique feature of the equipment in the Bethesda quarantine greenhouse is an automatic ventilating apparatus opened and closed by a series of thermostats which actuate a reversible electric motor. This was suggested by Eugene May, jr., and devised by

Harry Stevens, an electrical engineer of Washington, D. C. After some modifications and improvements now under way it is believed that this system of ventilation control may have wide application in greenhouse work. For quarantine houses, it obviates the necessity for frequent visits by workmen, with the attendant dangers of carrying infections.

The heating plant is also controlled by a thermostat of the ordinary commercial type, which is economical and likewise reduces to a minimum visits on the part of attendants. It may be said, in this connection, that only authorized attendants are allowed access to this quarantine greenhouse, which is kept locked at all times.

PLANT CAGES.

If only one lot of plants at a time were to be handled in quarantine, the problem would be comparatively simple: but different lots often from widely different parts of the world must be handled at the same time. These different lots of plants must, of course, be protected from each other. This difficulty is met by growing each importation first in a separate screened cage located in the isolation ward of the quarantine greenhouse.

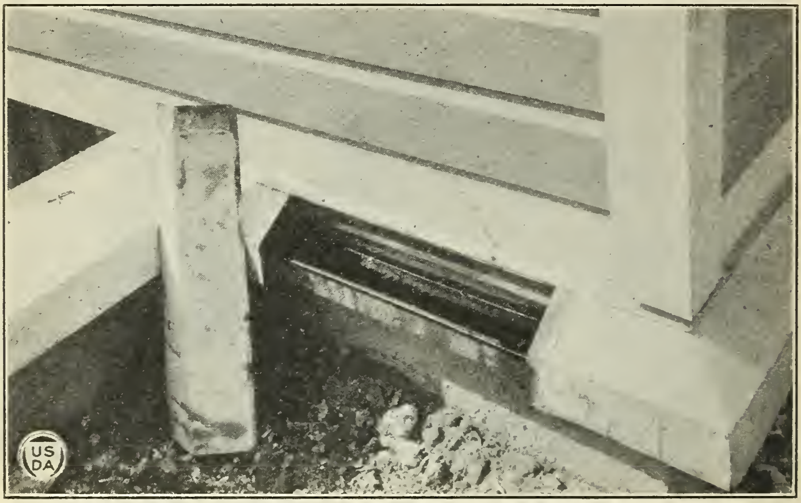

Fig. 7.—Oil moat surrounding the citrus quarantine greenhouse, with a section of the cover removed for inspection.

The cages now in use (fig. 10) are a recent invention. They are constructed entirely of metal, each provided with an oil moat, and so put together that by removing a few thumbscrews they can be taken apart and thoroughly sterilized before being used a second time.

The wooden cages formerly used had to be burned, as soon as they had served for a single lot of plants, to guard against carrying over some hidden pest. This was expensive and at the same time left open a chance that some new wood-destroying fungus might gain a foothold by producing spores before the cage was finally destroyed by burning.

The detail drawings (figs. 11, 12, and 13) show the method of construction of this all-metal, knockdown, insect-free cage specially designed for quarantine use. Those now in use were constructed in the mechanical shops of the United States Department of Agriculture, where many of the details of construction were igeniously worked out.

BENCHES.

Woodwork that is subject to decay and offers abundant places of concealment for insects has been avoided as far as possible in the construction of greenhouse benches. The benches in use (fig. 6) have metal sides and sectional tile bottoms. This type of construction permits thorough sterilization by live steam if necessary and is economical because the materials used are practically indestructible.

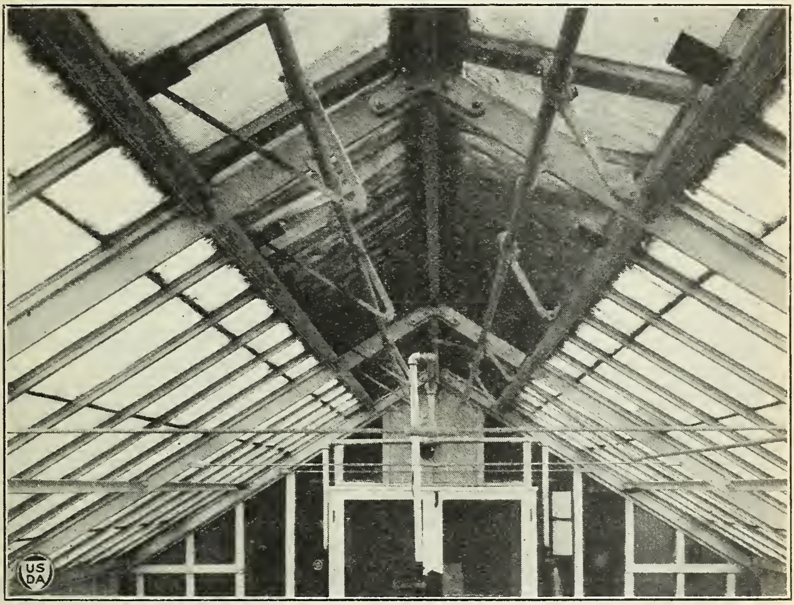

FIG. 8.—Screened ventilators of greenhouse made insect proof by a sliding gasket attached to the screen wire. The ventilator at the left is closed; that at the right, open.

PROPAGATION.

The foregoing descriptions cover chiefly the mechanical features found vitally important in growing citrus plants in quarantine so as to render them, or rather plants propagated from them, safe for later trial in citrus-growing territory. The propagation methods are fully as important.

Plant propagation must be carried on under conditions as nearly aseptic as it is possible to make them. This involves disinfection of clothing, tools, and person on each visit to the quarantine greenhouse. But the principal new feature in plant propagation evolved during the past few years by the writers is the system of double transfer of buds now practiced in handling imported citrus plants. No part of an original imported plant is ever permitted to leave quarantine alive.

PLANT REGENERATION THROUGH THE DOUBLE TRANSFER OF BUDS.

The original plants are held only until new growth is made suitable for furnishing budwood and until propagation can be made from this new growth. If this new growth is found to be entirely free from infection or infestation, buds are transferred (by budding or grafting) to new vigorous, home-grown stocks held in the isolation ward of the quarantine greenhouse. When these buds are safely established, the original plant and any adhering soil are put in a furnace and totally destroyed or sterilized. When the newly budded plant has made growth sufficient to furnish good budwood, a second transfer is made on new clean stocks, and if no disease has appeared in the meantime these last buds are admitted to the main section of the quarantine greenhouse. These first three stages may take a year or two, giving ample time in most cases for any latent disease to make its appearance. Nevertheless, there are two more stages before all quarantine restrictions are removed and the " regenerated " plants are eligible for field trial.

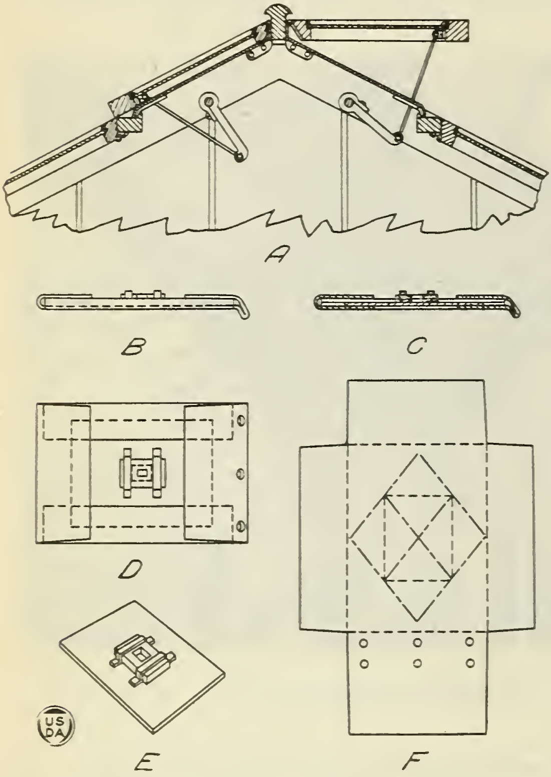

FIG. 9.—Insect-excluding device for use on greenhouse ventilators: *A*, Side view of the device in position on ventilators; *B*, side view of the trap; *C*, side sectional view of the trap; *D*, plane view of the trap; *E*, isometric view of the trap; *F*, sectional top view of the body of the trap prior to assembly.

RELEASE FROM QUARANTINE.

When released from the quarantine greenhouse (and release is made only on a certificate showing entire freedom from any sort of infestation), the plants are placed in a special screened compartment in one of the Washington greenhouses. This compartment is known as the "detention cage." When new growth has been made here which proves clean, the plants are allowed a place on the main bench of the greenhouse. Later they are repotted in freshly sterilized soil and are admitted to the main propagating house, when they become eligible for field trial. This propagating house is likewise fully equipped with insect-proof devices, so that when plants are finally ready for distribution they can be certified as entirely free from infestation or infection. No plants are sent out except when such a certificate can be given.

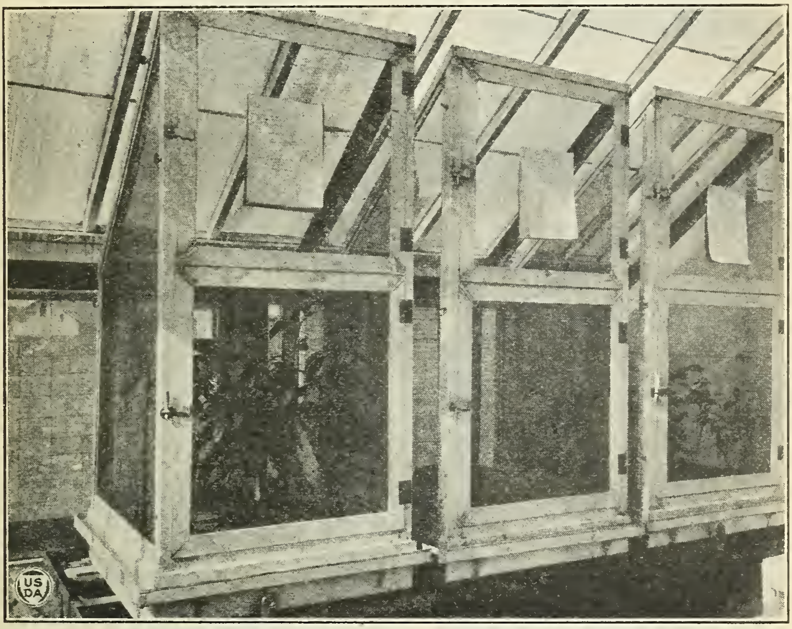

FIG. 10.—All-metal knockdown insect-free cages for growing recently imported citrus plants in the isolation ward of the citrus quarantine greenhouse.

There are thus five stages with at least two transfers of budwood before distribution. At any one of these stages a suspicious plant can be detected and destroyed. The same system in use for imported plants is employed, with some modifications, for handling citrus plants which it is desired to send from one State to another where mutual quarantine exists. The original plants are not passed for shipment, but newly propagated buds are sent out in such cases. Cooperative agreements with State quarantine officials designate the procedure to be followed in individual cases.

The procedure to be followed in handling imported citrus materials has been formulated into a set of regulations, a copy of which is kept conspicuously posted in the citrus quarantine greenhouse. These regulations are here summarized.

REGULATIONS.

SPECIAL PROCEDURE IN HANDLING IMPORTED CITRUS PLANTS, SCIONS, AND SEEDS.

(1) On receipt of a citrus importation, the Federal Horticultural Board sends, by agreement, the shipment unopened direct to the Bethesda greenhouse for inspection and propagation, to avoid exposure of other plants to infection.

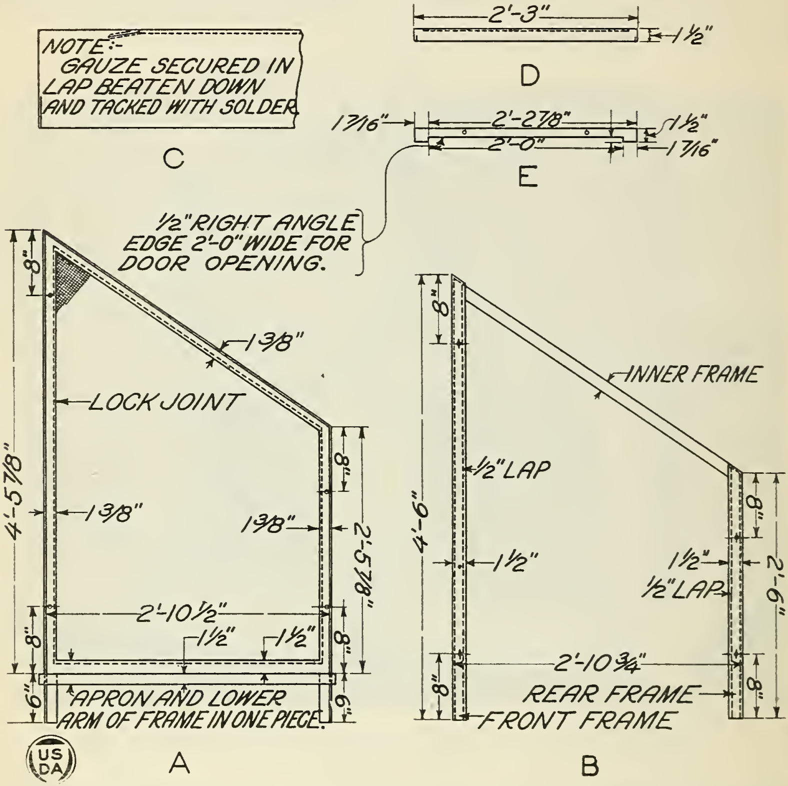

FIG. 11.—All-metal knockdown insect-proof plant cage: *A*, Inner or side frames; *B*, side view, showing outer frames; *C*, cross section of top frame, showing lock joint; *D*, cross section of top frame; *E*, front end of top frame.

(2) Original packages or containers must be burned after unpacking, preserving all marks, labels, tags, etc., after sterilization of same.

(3) The plants when potted are placed in a Wardian case or sterile cage especially screened and kept thus isolated from other plants in the isolation ward of the greenhouse. Scions and seeds are kept similarly isolated when first propagated.

(4) As soon as new growth has been made of sufficient size to furnish budwood (if no canker or other disease has been observed), buds are taken and inserted on new clean stocks, and these new plants

are grown on the bench in the isolation ward of the greenhouse. If, however, canker or other unusual disease should be found, the affected material is destroyed by burning.

(5) As soon as the newly budded plants are safely established, the original plants and the case containing them, with pots and soil, are destroyed (or sterilized) by burning in a furnace.

(6) A second transfer of buds is made from the first plants budded from the originals, and these budded plants are admitted to the main greenhouse if no disease has appeared in the isolation ward during the period of first propagation.

(7) After a period of growth and inspection in the main house (at least six months) inspection is made by the Federal Horticultural Board inspectors, and if the plants are found entirely free from infestation or infection they are released for propagation in the special insect-free detention portion of the Washington citrus greenhouse.

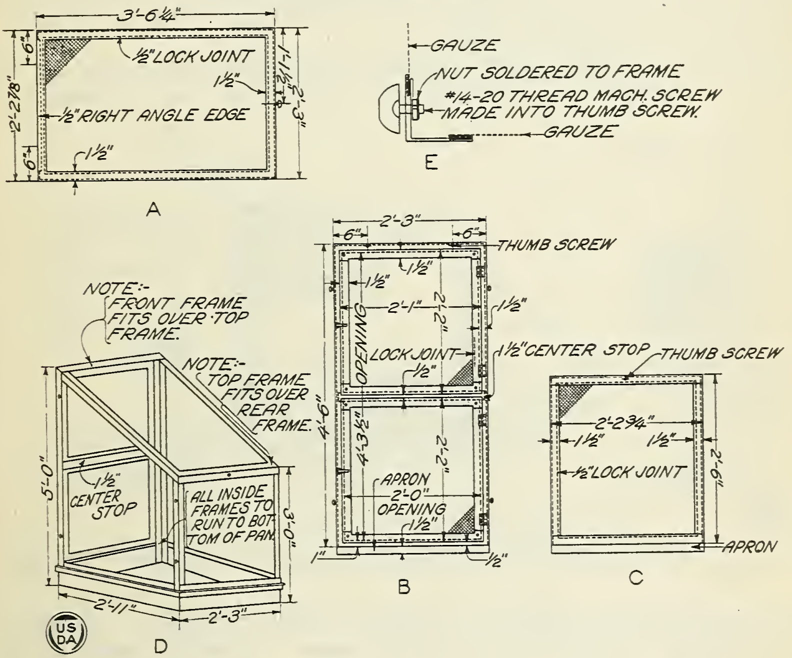

FIG. 12.—All-metal knockdown insect-proof plant cage: *A*, Plan of top frame; *B*, outer or front frame and doors; *C*, outer or rear frames; *D*, profile of cage; *E*, detail plan of frames, showing thumbscrews holding the frames secure.

(8) At all times, persons working in the quarantine house or others attending or inspecting are required to wear special suits and shoes that can be readily disinfected. A solution of mercuric chlorid (1–1,000) must be used before entering and before leaving the greenhouse and after touching any pot or plant in the isolation ward of the greenhouse or before leaving this part of the house to enter the main greenhouse.

(9) All tools used in greenhouse work must be sterilized frequently with mercuric-chlorid solution (after each handling); frequent in-

spections should be made of the oil cups, oil moat, and screens to maintain effective insect barriers.

(10) No visitors are to be admitted unless attended by the person in charge of the quarantine house or a specially designated assistant. Visitors shall be excluded from the isolation ward except under circumstances of unusual necessity. The house shall be kept locked at all times.

OBJECT OF THIS PROCEDURE.

The object and spirit of this whole procedure is to provide that no portion—root, branch, bark, bud, or seed—of the original imported plant shall ever be released from quarantine, all parts of the original plant and the adhering soil being destroyed or sterilized by fire after new, clean plants have been obtained by fresh propagation on healthy home-grown stocks.

There are, of course, some details of procedure not covered in the foregoing regulations. Fumigation is practiced when necessary if the plants are in a condition to withstand such treatment. Spraying or dusting is resorted to in individual cases. Whenever possible, the new growth on recently imported plants is isolated from the old wood by the application of adhesive bands to prevent the migration of any insect pests present.

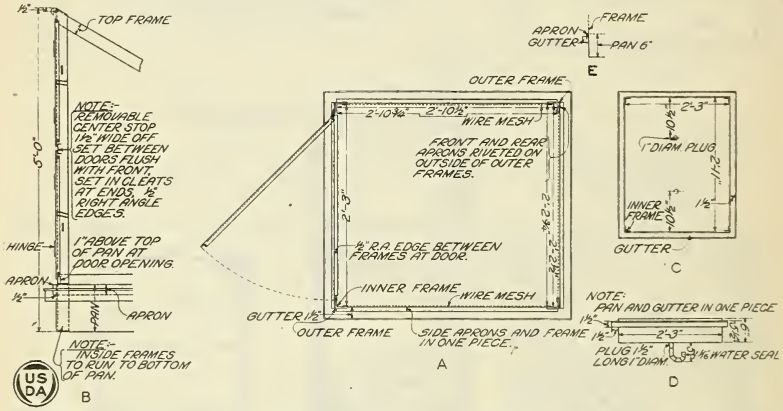
½"
TOP FRAME
5'-0"
NOTE:-
REMOVABLE CENTER STOP 1½" WIDE OFF SET BETWEEN DOORS FLUSH WITH FRONT, SET IN CLEATS AT ENDS, ½" RIGHT ANGLE EDGES.
HINGE
1" ABOVE TOP OF PAN AT DOOR OPENING.
APRON
½"
APRON
NOTE:-
INSIDE FRAMES TO RUN TO BOTTOM OF PAN.
B
FRAME
APRON
GUTTER
PAN 6"
E
OUTER FRAME
2'-10¾"
2'-10½"
WIRE MESH
FRONT AND REAR APRONS RIVETED ON OUTSIDE OF OUTER FRAMES.
2'-3"
2'-2¾"
2'-2½"
½" R.A. EDGE BETWEEN FRAMES AT DOOR.
INNER FRAME
WIRE MESH
GUTTER 1½"
OUTER FRAME
SIDE APRONS AND FRAME IN ONE PIECE.
A
2'-3"
10½"
1" DIAM. PLUG
2'-1½"
INNER FRAME
10½"
1½"
GUTTER
C
NOTE:
PAN AND GUTTER IN ONE PIECE
1½"
1½"
2'-3"
6"
PLUG 1½" LONG 1" DIAM.
WATER SEAL
D

FIG. 13.—All-metal knockdown insect-proof plant cage: *A*, Plan showing frames, apron, and gutter; *B*, section of front; *C*, plan of pan and gutter; *D*, outline view of pan.

CONCLUSION.

As a result of the equipment and procedure here described, the citrus quarantine greenhouse instead of being a sort of plant "pest house," as it might easily become, has proved to be as near absolutely clean in a horticultural sense as it is humanly possible to make it. Repeated inspections of the most searching character have failed to disclose evidence of any kind indicating the presence of insect, fungous, or bacterial pests on the newly propagated plants. The successful combating of insect infestation is considered the essential preliminary step, and this is now proved to be merely a matter of mechanical detail. The extra cost of such insect-proof construction is hardly more than the cost of constantly fighting insects and suffering losses from their depredations. No system is proof against personal carelessness or ignorance, and the success so far obtained is doubtless in large measure due to the personal interest taken by the assistants employed in this work. As modern medicine has developed a system of aseptic surgery, so must modern horticulture recognize aseptic propagation, in a liberal sense, as possible and vitally necessary in dealing with imported plants.

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